

the special action of apple oxidase on tannic acid is due to the presence of a phosphatic sensitiser, it would be a feebler oxidase of the same type. Carrot and parsnip oxidases are a grade feebler, but still react to guaiacum in the absence of a peroxide. Malt diastase is still weaker, and papain feebler still, while pepsin may show a weak "peroxidase" reaction with guaiacum, but not any other oxidase action.

*The Fixation of Arsenic by the Brain after Intravenous
Injections of Salvarsan.*

By JAMES MCINTOSH, Beit Memorial Research Fellow, and PAUL FILDES,
Assistant Bacteriologist to the London Hospital.

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(From the Bacteriological Laboratory of the London Hospital.)

During the period of probation of salvarsan as an anti-syphilitic remedy, a number of toxic phenomena were reported which led to the belief that this drug had particular neurotropic properties, and was therefore to be used with the greatest circumspection. These fears were very largely founded upon the well known effect of the related drug atoxyl in producing optic atrophy. Subsequent experience has, however, shown that the supposed neurotropic action of salvarsan was due to certain technical errors in its administration.

In 1911 we published (1) an observation which combated the view that salvarsan had neurotropic qualities. We submitted the organs of an infant who died after administration of salvarsan to Dr. W. H. Willcox for analysis, and he reported to us that the brain in this case contained no arsenic, although considerable quantities were present in other organs. We then applied the law of Ehrlich, "*corpora non agunt nisi fixata*," and argued that, since the brain was free from arsenic, salvarsan could have no neurotropic action.

Exactly similar conclusions were arrived at by Ullmann in 1913 (2). In the course of a very extensive investigation upon the distribution of arsenic in the body after salvarsan injections, he made it quite clear that the brain never contained more than traces of arsenic, and "this fact was evidence against the neurotropic action of salvarsan." Similarly, Morel and

Mouriquand (3) found the brain and cord in five animal experiments to be free from arsenic, although other organs contained much. They also concluded that salvarsan has no neurotropic effect. On the other hand, Mouneyrat (4), after the injection of salvarsan into animals, found arsenic "in very appreciable quantities in the liver, muscles, and brain, distinctly more than the infinitesimal amounts met with in the control animals, which had had the same food but no injection." Thus Mouneyrat considered that salvarsan was "particularly neurotropic." In criticism of this paper it must be stated that the author gives no evidence of having made quantitative estimations, by which he might have discovered that the liver and kidney, for example, contained vastly more arsenic than the brain. Further, the fact that he was able to demonstrate arsenic in the brains of normal animals must lead to a certain suspicion of his other results.

The observations of Ullmann, Morel and Mouriquand and ourselves were accepted by Ehrlich (5) as showing that salvarsan has no "Vorliebe" for the brain.

As a result of subsequent investigations upon the minute anatomy of the cerebral vessels, however, we were not entirely satisfied that the deductions we had drawn were correct.

It appeared that absence of arsenic from the brain might be due to two distinct factors. Firstly, the arsenic might not be "fixed" by the brain as already suggested, or, secondly, it might not gain access to the brain, owing to some peculiarity of the cerebral vessels. In order to test this possibility we conducted experiments *in vitro*, applying neosalvarsan directly to fresh brain substance, and then found that neosalvarsan was in fact "fixed" by the brain.

Experiment 1.—100 gm. of fresh human brain and 100 gm. of human liver were minced and placed in two separate glass bottles of 1000 c.c. capacity. These bottles were filled with saline solution, shaken, allowed to stand, and the supernatant fluid removed. The tissue was thus freed from excess of blood; 500 c.c. of saline solution, containing 0.15 gm. of neosalvarsan, were then added to each, and the bottles were shaken for one hour. At the end of this time the supernatant fluid was removed by decantation and the bottles filled with saline solution eight times, shaking on each occasion and removing the washings by decantation. By this method it was hoped that all "unfixed" neosalvarsan would be removed from the tissues.

Estimations of the amount of neosalvarsan in the washed tissue, the supernatant fluid after fixation, and the final washing fluid were then made.

In every case the material to be tested was heated with concentrated nitric

acid and sulphuric acid, the residue extracted with water and tested in the Marsh apparatus. The technique employed was that advocated by Chittenden and Donaldson (6). The rings of arsenic obtained in this way were compared with a standard series of rings made from known weights of neosalvarsan, and the amount of arsenic was expressed as grammes of neosalvarsan. In no case was any attempt made to obtain accurate quantitative results, and our figures merely indicate roughly whether a particular sample contained much or little neosalvarsan :—

		Gramme of neosalvarsan.
Moist brain residue after washing, 100 gm.	contained	0·004
Supernatant fluid after fixation with brain, 500 c.c.	„	0·1
Final washing fluid.....	„	0
Moist liver residue after washing, 100 gm.....	„	0·015
Supernatant fluid after fixation with liver, 500 c.c....	„	0·1
Final washing fluid.....	„	0

From this experiment it appears that brain substance will fix considerable quantities of neosalvarsan *in vitro*.

Experiment 2.—Repetition of Experiment 1.

		Gramme of neosalvarsan.
Moist brain residue after washing, 100 gm.	contained	0·01
Supernatant fluid after fixation, 500 c.c.....	„	0·05
Final washing fluid	„	0
Moist liver residue after washing, 100 gm.	„	0·01
Supernatant fluid after fixation, 500 c.c.....	„	0·05
Final washing fluid	„	0

Experiment 3.—Repetition of Experiment 1, but twice the quantity of brain and liver used, viz., 200 gm. The results were similar to those obtained in the other experiment, although the brain substance appeared to fix more neosalvarsan than did the liver, thus :—

		Gramme of neosalvarsan.
Moist brain residue, 200 gm. contained		0·02
Moist liver residue, 200 gm. „		0·004

It may be objected to these experiments, that the arsenic was merely entangled mechanically with the minced tissues and was not chemically fixed; we therefore repeated the experiment after replacing the animal tissues with fragments of Doulton filter (asbestos) and finest vegetable charcoal.

Experiment 4.—50 grm. of broken-up filter candle were placed into 250 c.c. of saline solution and 0·075 grm. of neosalvarsan added. Further technique as in Experiment 1.

Result.—

		Gramme of neosalvarsan.
Moist filter residue after washing, 50 grm.....	contained	0
Supernatant fluid after fixation, 250 c.c.....	„	0·04
Final washing fluid	„	0

Experiment 5.—Repetition of Experiment 4, but with finest Venetian charcoal instead of Doulton filter. Air was removed from the charcoal by boiling and the use of the vacuum pump—0·15 grm. of neosalvarsan were added.

		Gramme of neosalvarsan.
Moist charcoal residue after washing, 50 grm....	contained	0·001
Supernatant fluid after fixation, 250 c.c.	„	0·11
Final washing fluid	„	0

Although a certain degree of absorption of the neosalvarsan was observed in the case of charcoal, it was not so great as with liver and brain tissue, and this fact, taken with the absence of fixation by the filter, suggests that the presence of arsenic in the latter after washing was a true combination and not a purely mechanical phenomenon.

The conclusion may therefore be drawn that the absence of arsenic from the brain after intravenous injection of salvarsan is not due to a lack of affinity between the drug and the brain, but to an inability of the drug to penetrate into the proper brain substance.

As is well known, arsenic is not usually found in the cerebrospinal fluid after intravenous injections of salvarsan, and this has been suggested as an explanation of the lack of effect of this drug upon certain syphilitic affections of the brain. On other data, however, we have formed the opinion that the inability of arsenic to penetrate the brain has no relation to its absence from the cerebrospinal fluid, but is due to a peculiarity of the cerebral capillaries. If, however, neosalvarsan is introduced directly into this fluid by lumbar puncture, then, as shown by Wechsellmann, marked toxic symptoms appear.

Experiment 6. To Demonstrate the Toxicity of Neosalvarsan to the Nervous System.—In a typical experiment two rabbits were used in addition to a control.

0·05 grm. of neosalvarsan were dissolved in 10 c.c. of saline solution, and

of this solution 0·2 c.c. were injected into the cerebrospinal fluid of a rabbit weighing 500 gm., through the posterior occipito-atlantoid membrane. The effect of the injection was to produce convulsions immediately, and death the next day. A second rabbit (800 gm.) received 0·1 c.c. and was found to be paralysed on the following day. As a control, 0·2 c.c. of saline solution was shown to be innocuous. It thus follows that about 0·0005 gm. of neosalvarsan is the toxic dose to a rabbit of 800 gm. weight when injected into the cerebrospinal fluid. It may be assumed that the drug is rapidly absorbed from the cerebrospinal fluid into the brain and is there fixed as in our experiment conducted *in vitro*. Thus salvarsan may be said to be as much "organotropic" to the brain as to the liver, but this effect is not apparent after its therapeutic administration in the ordinary manner.

The inability of salvarsan to penetrate into the brain explains the lack of success which often attends the treatment of syphilitic lesions of the brain, and in particular the parenchymatous varieties (dementia paralytica and tabes dorsalis).

We next considered whether this inability might be due, in some measure, to the rapid fixation of the drug by the liver and kidneys and its removal from the blood.

We have indeed found, experimentally, that the blood is practically free from arsenic two days after an injection, as shown in the following experiment:—

Experiment 7.—Four rabbits were injected intravenously, each with 0·15 gm. of neosalvarsan. They were killed 3, 6, 24, and 72 hours after the injection. Specimens of blood were collected and the animal transfused with saline to remove the blood from the organs. The blood and organs contained the following quantities of neosalvarsan:—

	Rabbit A, 3 hrs.	Rabbit B, 6 hrs.	Rabbit C, 24 hrs.	Rabbit D, 72 hrs.
Brain (<i>ca.</i> 10 gm.)	? trace	Grammes of neosalvarsan.		
Liver (10 gm.)	0·0005	? trace	0	0
Blood (25 c.c.)	0·003	0·00075	0·0012	0·00025
		0·0006	0·0004	0

We next endeavoured to satisfy the affinity of the liver and kidneys for arsenic by repeated injections of neosalvarsan, in the hope that a sufficient content would be maintained in the blood to allow of penetration into the brain.

Experiment 8.—Three rabbits were injected, each twice daily for four days

with 0·05 gram. of neosalvarsan. They were killed 12, 36, and 60 hours after the last dose and transfused as before.

The following results were obtained :—

	Rabbit A, 12 hrs.	Rabbit B, 36 hrs.	Rabbit C, 60 hrs.
	Grammes of neosalvarsan.		
Brain (ca. 10 gm.)	0·0001	0·00004	0
Liver (10 gm.)	0·0005	0·00075	0·00075
Blood (25 c.c.)	0·0003	0·0001	0

Experiment 9.—Repetition of Experiment 8, but all the animals killed 24 hours after the last dose.

	Rabbit A.	Rabbit B.	Rabbit C.
	Grammes of neosalvarsan.		
Brain (ca. 10 gm.)	0	0	0
Liver (10 gm.)	0·00012	0·0001	0·0005
Blood (25 c.c.)	0	0·0001	0

From these experiments it appears that no definite penetration of the drug into the brain can be obtained by a repetition of the injections, although in one case a small quantity was observed 12 hours after the last dose.

Similar results were obtained with Prof. Ehrlich's new copper-salvarsan combination (k_3).

Experiment 10.—Technique as in Experiment 8. The dose of k_3 was 0·02 gram. and the animals were killed 24 hours after the last injection.

	Rabbit A.	Rabbit B.
	Grammes of neosalvarsan.	
Brain (ca. 10 gm.).....	0	0
Liver (10 gm.)	0·00002	0·0005
Blood (25 c.c.).....	0	0

Conclusions.

1. After intravenous injections of salvarsan and neosalvarsan in man and animals no arsenic can be found in the brain.

2. This phenomenon is not due to a lack of affinity between the brain and the drugs, but to an inability on the part of the drugs to penetrate into the substance of the brain.

3. Fixation of arsenic by the brain occurs as readily as by the liver, as shown by experiments *in vitro* and the toxic effects of intrathecal injections.

4. Penetration of neosalvarsan into the brain cannot be obtained even by frequently repeated intravenous injections.

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The Production of Anthocyanins and Anthocyanidins.—Part II.

By ARTHUR ERNEST EVEREST, M.Sc., Ph.D. (Lecturer in Chemistry,
University College, Reading).

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The author of the present paper* and other investigators† have concluded as the result of their investigations that the red pigments obtained as the result of careful reduction of the yellow flavonol derivatives are identical with the natural anthocyanins of plants. Prof. Willstätter, on the other hand, holds‡ that these artificially produced red pigments are different from the natural anthocyanins.§

That the point at issue is important may be judged from the facts that, the complete synthesis of flavonol derivatives having been realised by Kostanecki, the production of anthocyanins from them as described by the author (*loc. cit.*), Watson and Sen,|| and Combes,¶ completes the synthetic production of these compounds. Moreover, the production of anthocyan glucosides—anthocyanins

* 'Roy. Soc. Proc.,' B, vol. 87, p. 444 (1914).

† *Cf. ibid.*, p. 446.

‡ 'Sitzungsber. K. Akad. Wiss. Berlin,' vol. 12, p. 402 (1914).

§ Since the present paper was forwarded for publication, Wheldale and Bassett, 'J. Genetics,' vol. 4, p. 103 (1914), have published a criticism of the author's views, in which they also take up a similar attitude.

|| 'Chem. Soc. Journ.,' vol. 105, p. 389 (1914).

¶ 'Compt. Rend.,' vol. 157, pp. 1002 and 1454.